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Stelar Anatomy of Six Species of Lycopodium[1]

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A review of the literature indicates that only limited studies have been made on the stelar patterns of the club mosses and that little has been attempted in comparative investigations. Holloway (1916) found that the vascular cylinders of New Zealand lycopods exhibited specific stelar patterns. He thought that the cortical sclerenchyma affected stelar arrangement and that the presence of this tissue could be responsible for imparting rigidity and lateral pressures to the stele. This rigidity and lateral pressure support the stele in such a way that the xylem elements become more definitely arranged in groups or bands.

Later, Holloway (1919) concluded that there were two basic patterns in which the xylem elements were arranged; these patterns also appeared to be associated with the leaf trace system: (*i*) the mixed type in which the leaf trace system extends beyond the cauline vascular cylinder and (*ii*) the banded or parallel type in which the vascular cylinder becomes strongly developed and the leaf traces affix themselves to it. He interpreted the banded type as a specialization of a radial pattern that was caused by a restriction on the stem in monopodial branching. Holloway also associated stelar patterns with prothallus type: short-lived prothalli were characteristic of the mixed type of vascular cylinder and long-lived prothalli of the banded pattern. He noted that these differences were exhibited in all the species of *Lycopodium*

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[1] This study represents a portion of a doctoral dissertation submitted to the Graduate School of St. Bonaventure University, St. Bonaventure, N. Y. Appreciation is extended to Dr. Alfred F. Finocchio for advice during the course of the research.

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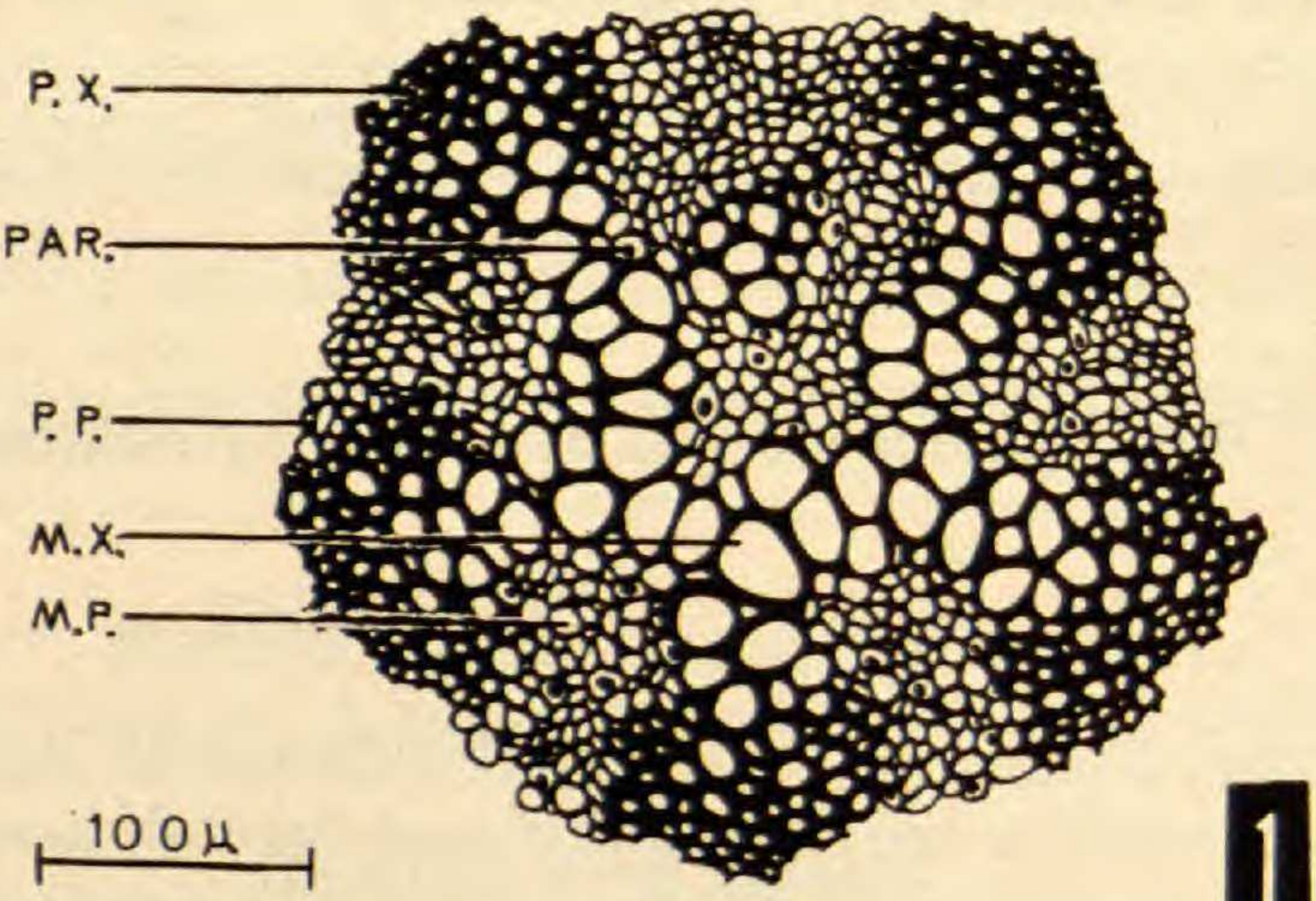

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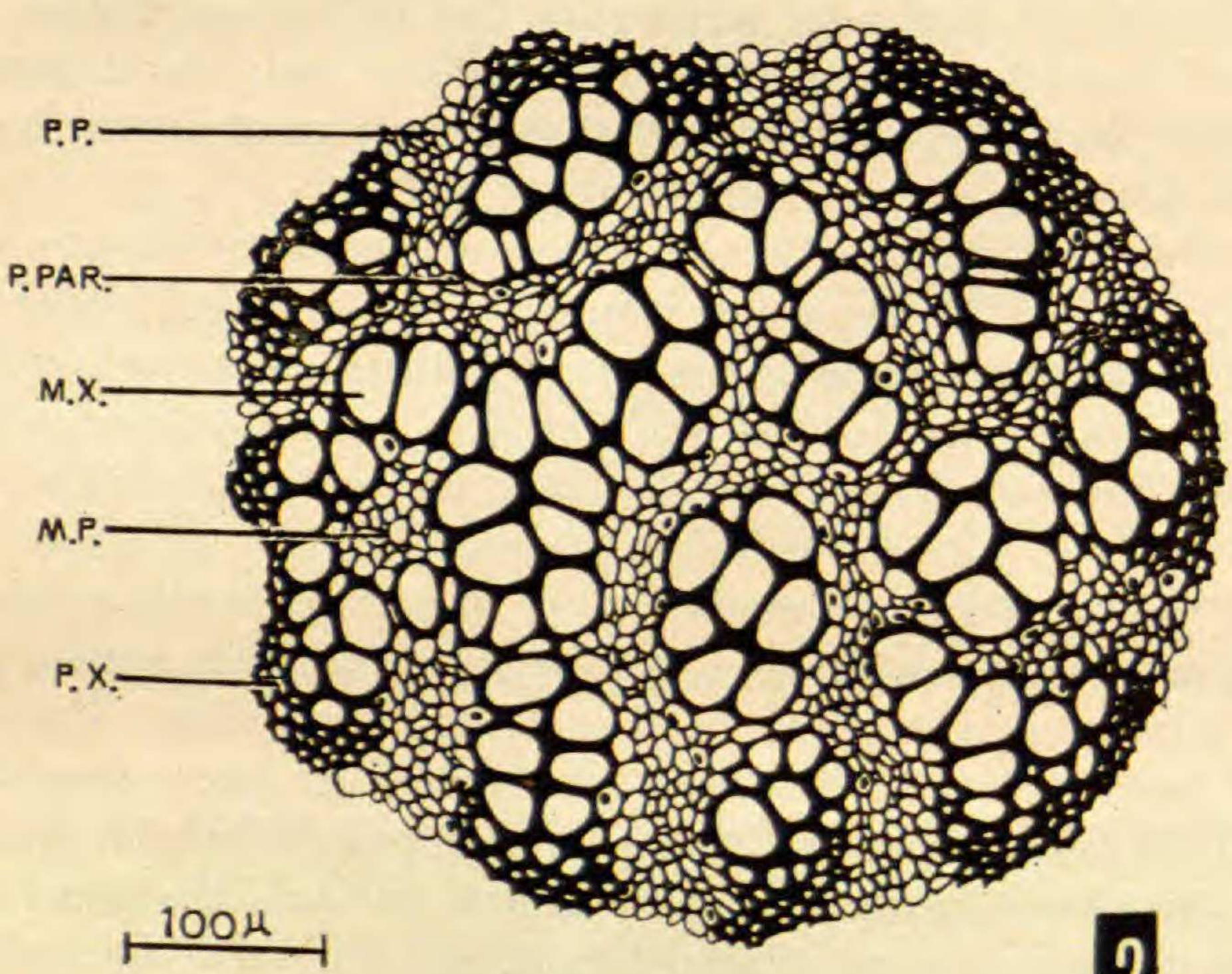

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TRACINGS OF COMPOSITE PHOTOGRAPHS OF THE VASCULAR CYLINDERS OF LYCOPODIUM LUCIDULUM AND L. ANNOTINUM. The abbreviations are: m.p. = METAPHLOEM, m.x. = METAXYLEM, p.par. = PHLOEM PARENCHYMA, p.p. = PROTOPHLOEM, and p.x. = PROTOXYLEM.

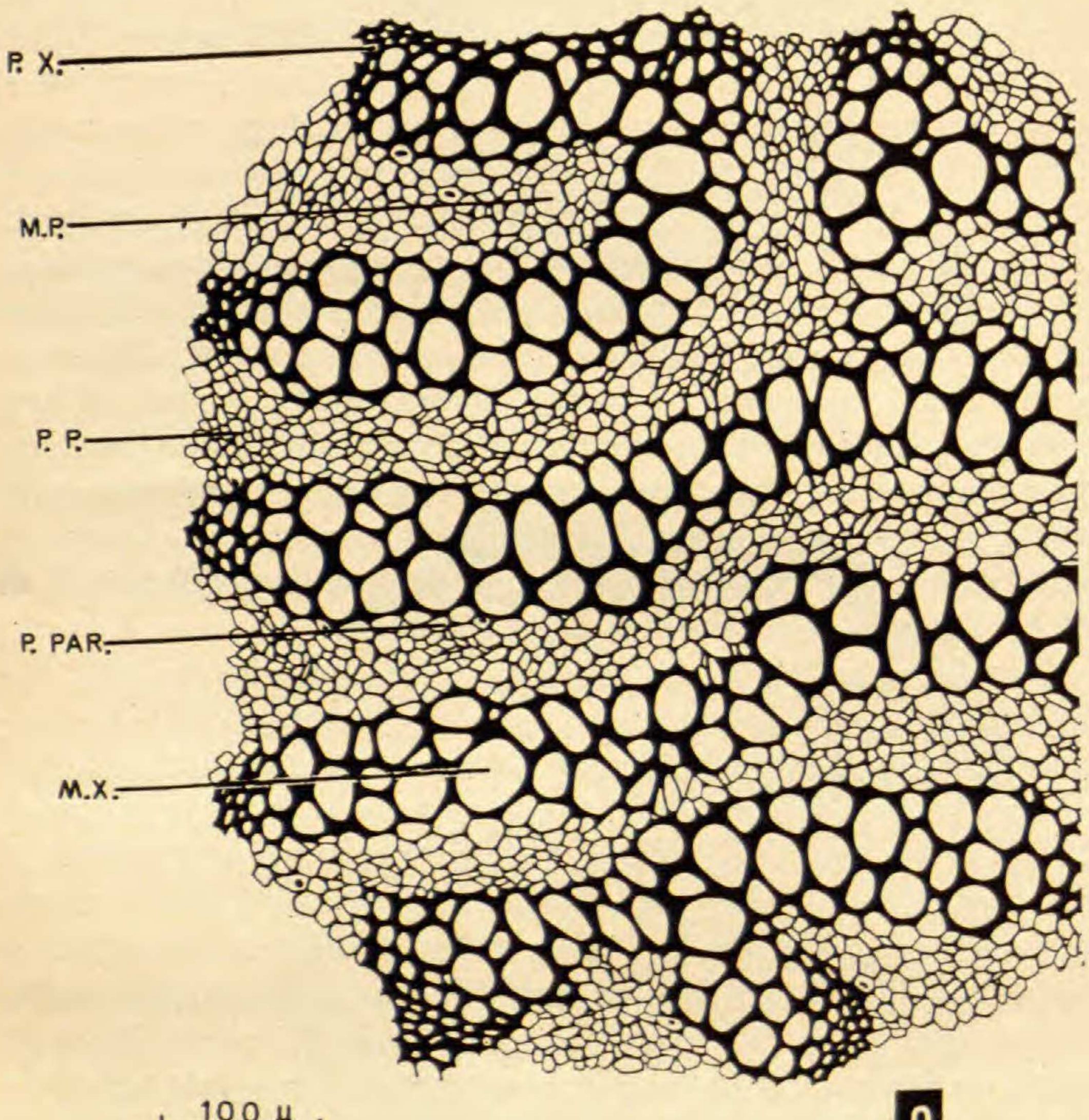
which he studied, regardless of whether their growth habit was vertical or horizontal.

This study was undertaken to examine additional species of *Lycopodium*. A comparative investigation of the transverse sections of stems of six species found in the northeastern United States revealed similar stelar patterns in addition to several other observations. The species collected in southern Cattaraugus County, New York (Cardillo, 1967) were: *Lycopodium lucidulum* Michx., *L. flabelliforme* (Fernald) Blanchard, *L. tristachyum* Pursh, *L. clavatum* L., *L. annotinum* L. var. *annotinum*, and *L. obscurum* L.

First, stem sections of the same species taken at various distances from the apex were compared. These showed slight differences which could be attributed to age (Bower, 1935). It was evident, however, that such differences did not alter the basic stelar pattern. For comparative study transverse sections were taken from subapical regions of the main stems where maturation was known to have occurred. This would rule out the minimal differences in stelar patterns attributed to age.

Tissues were killed and fixed by freeze substitution and dehydrated in cold absolute ethyl alcohol (Jensen, 1962). Iodine–potassium iodide served to detect phloem and phloem parenchyma; phloroglucinol-HCl was used to detect xylem (Roberts & Herty, 1934). Since this investigation dealt with a comparative study of mature stelar patterns and the cortical sclerenchyma was not under study except for its effect on supporting vascular tissue in the living plant, the cortical material was removed just before killing and fixing. Thus, steles were processed instead of entire stem sections. This facilitated histological procedures and gave more accurate results. Fisher Tissuemat (melting pt. 56.5° C) was used for embedding. Sections were stained with basic fuchsin and counterstained with fast green so that a cellular inclusion giving a positive Feulgen reaction could then be identified as a nucleus (Jensen, 1962).

Since cell diameters ranged from 3 μ to 50 μ, it was necessary to examine each transverse section at a magnification of 430 $\times$ or higher. In order to make a comparative study of cellular size and



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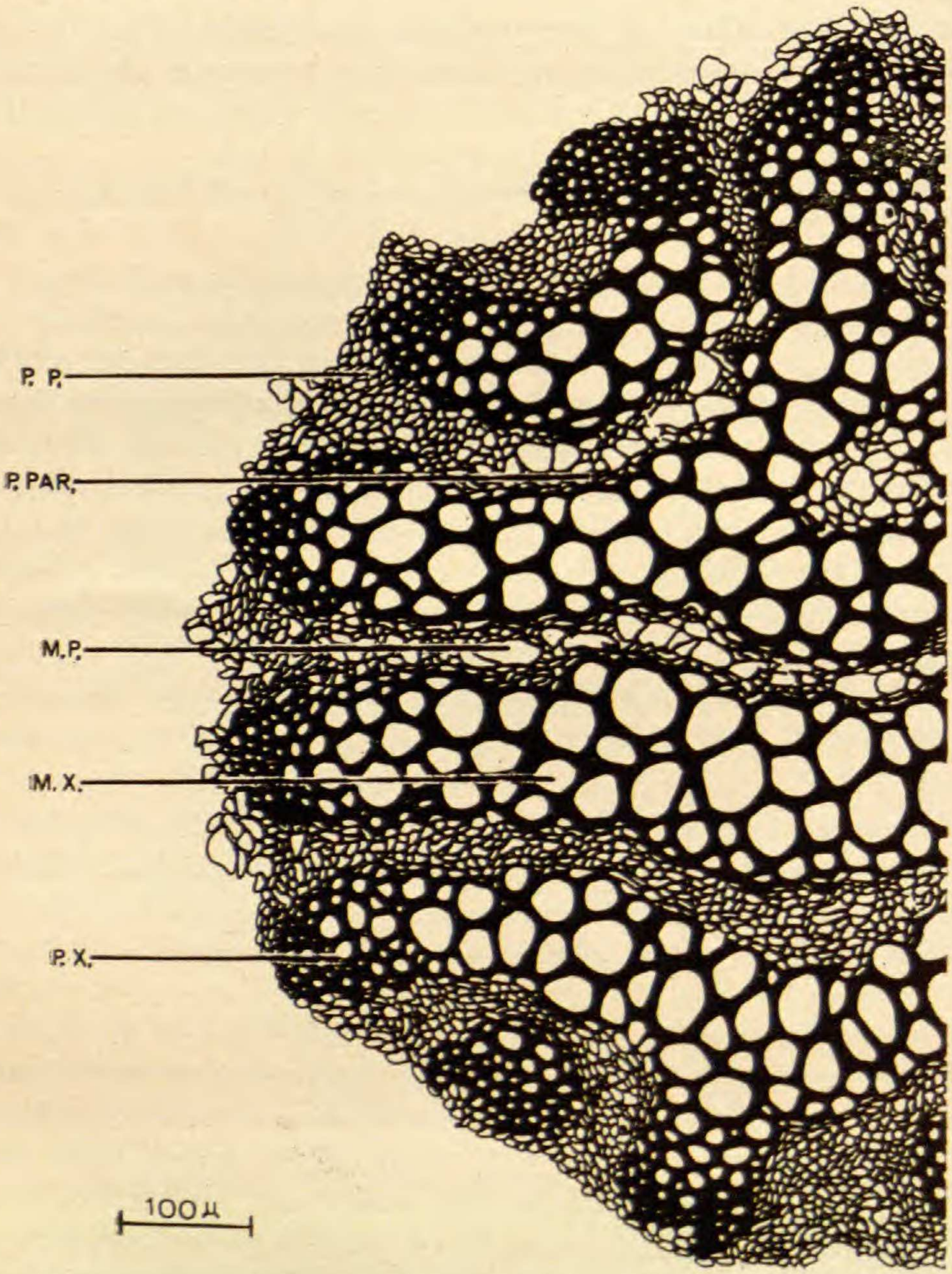
TRACING OF A COMPOSITE PHOTOGRAPH OF THE VASCULAR CYLINDER OF LYCOPODIUM OBSCURUM. For abbreviations see *Plate* 9.

shape, illustrations of these sections were made. *Figs. 1–6* are accurate drawings that were traced from composite photographs of each stele. Such composite photographs were compiled so that the details of very small cells would not be lost.

Differences not previously reported were revealed in this study. The results agree with Wardlaw's (1965) statement that differences of detail are only exposed in a comparative study of species in the same genus. *Table I* gives a comparative description of these details. These results also substantiate Bower's (1935) description of the stele of *Lycopodium* in that the xylem tracts are not distinct from one another for any great distance along the stem. The xylem structure anastomoses and there is a continuous invagination of the phloem and conjunctive parenchyma. In some areas of the plant, the stele with respect to stem diameter enlarges, but a relatively constant surface proportion of living tissue to adjoining dead tracheids is always maintained.

Very few nuclei were detected in any one section. Macerated preparations and longitudinal sections of the stele showed sieve cells and phloem parenchyma cells of unusual length. These cells also exhibit long and considerably vacuolated nuclei. Whether or not cells containing these nuclei were protophloem, phloem parenchyma, or pericycle could not be determined. No attempt was made to identify these cells in macerated preparations; in longitudinal sections the only way to identify the cell type is by position in respect to the xylem and the sieve areas located on the walls of the phloem and protophloem. Cells of the central portion of each phloem band were enucleate, but nuclei were detected in cells on the periphery of these bands. In longitudinal section, these cells of the central portion appear thin walled and are marked with sieve areas. Cells of the protophloem located at the periphery of the stele and intermitent with protoxylem bear similar markings. These cells contain nuclei and some also contain abundant cytoplasmic inclusions which were detected in sections stained with Conant's Quadruple Stain. Results with the periodic acid–Schiff reaction also show cytoplasmic inclusions, but these tissues produce a negative Feulgen reaction; evidently the cellular contents



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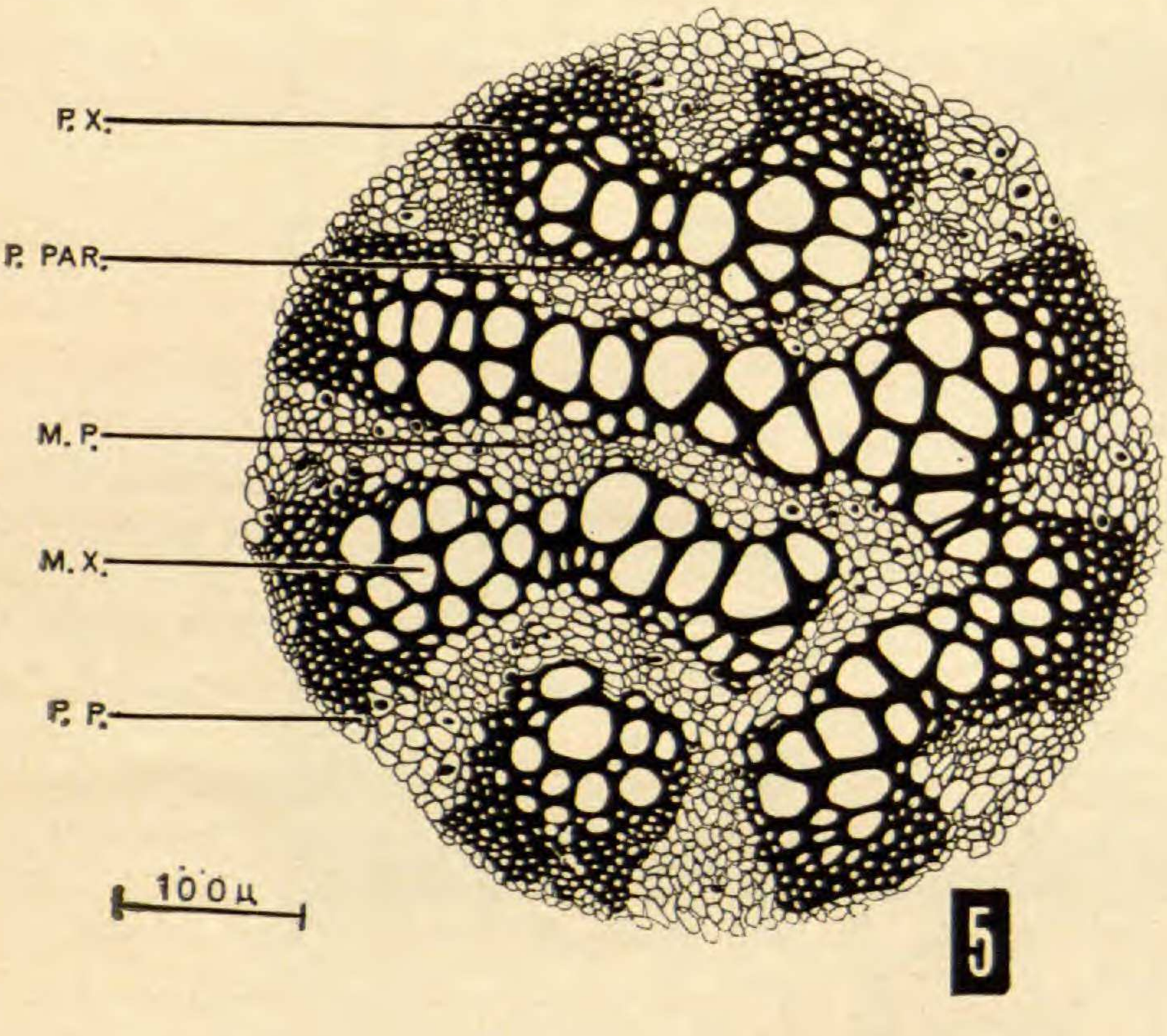
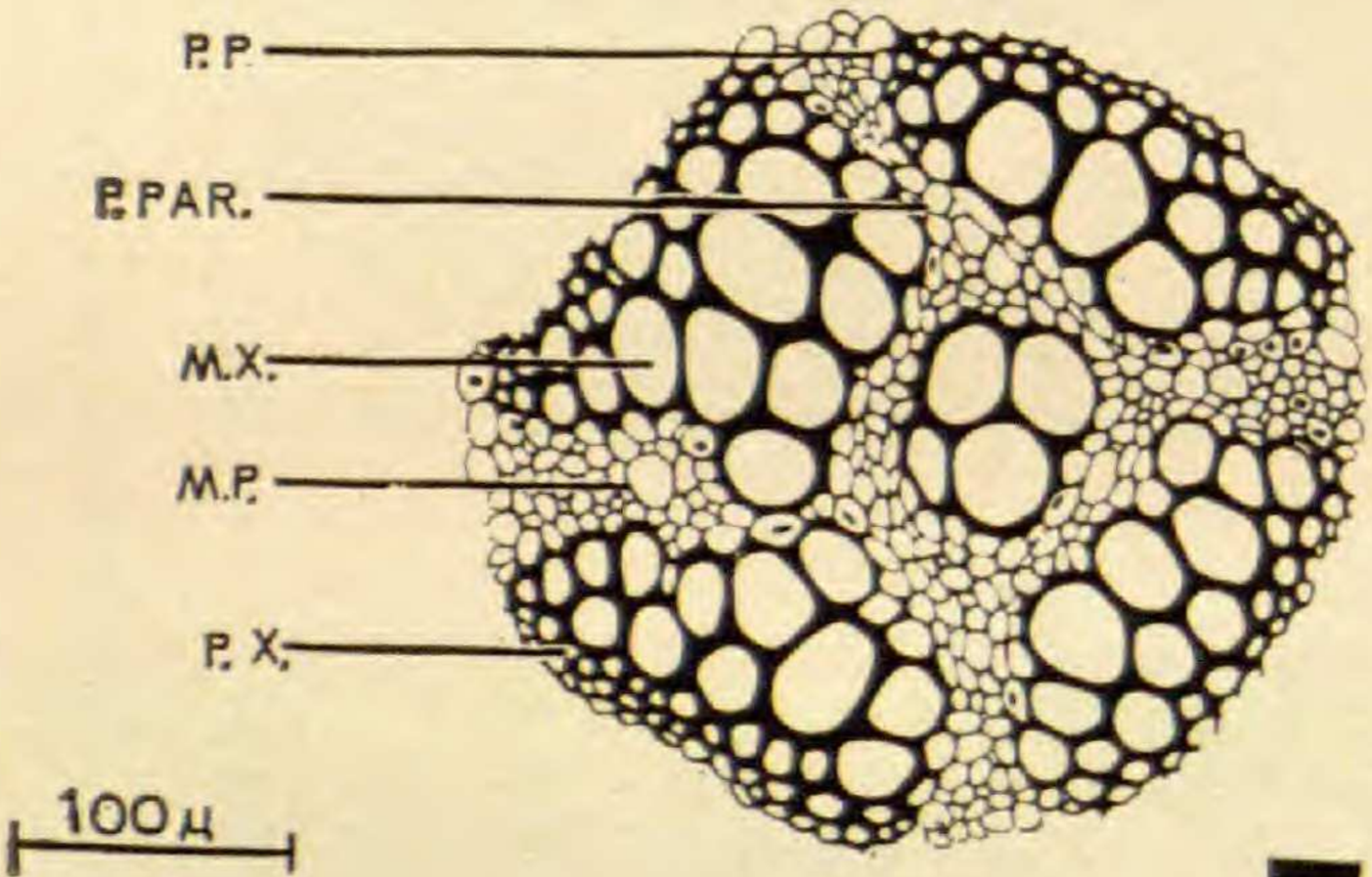
TRACING OF A COMPOSITE PHOTOGRAPH OF THE VASCULAR CYLINDER OF LYCOPODIUM FLABELLIFORME. For abbreviations see *Plate* 9.

TABLE I. COMPARISON OF STELAR FEATURES OF SPECIES OF LYCOPODIUM

Species	*Approx. size of stele*	*Pattern*	*Range in tracheid diameters*[1] *Protoxylem*	*Metaxylem*	*Description of nontracheary elements*
L. lucidulum, *Fig. 1*	350 μ	radial	5–8 μ	7–30 μ	small cells all similar in size
L. annotinum var. annotinum, *Fig. 2*	500 μ	banded	4–8 μ	10–40 μ	small cells; differ slightly in size
L. obscurum, *Fig. 3*	700 μ	banded	3–7 μ	7–30 μ	slightly larger cells; slight differentiation in size
L. flabelliforme, *Fig. 4*	950 μ	banded	4–9 μ	12–56 μ	marked differentiation in size of cells in central bands
L. clavatum, *Fig. 5*	525 μ	banded	3–8 μ	10–50 μ	noticeable differentiation in cell size
L. tristachyum, *Fig. 6*	325 μ	banded	6–8 μ	10–45 μ	slight differentiation in cell size

[1] Data represent a statistical analysis (Cardillo, 1966).





TRACINGS OF COMPOSITE PHOTOGRAPHS OF THE VASCULAR CYLINDERS OF LYCOPODIUM CLAVATUM AND L. TRISTACHYUM. For abbreviations see *Plate* 9.

are not nuclear material.

This investigation shows that in addition to the basic stelar patterns known to exist in the genus *Lycopodium*, as described by Holloway (1919), there is a difference in size, shape and orientation of the xylem and phloem tissue in the six species herein studied. The differences are characteristic of each species, and in anatomical studies they could be used as criteria for species identification.

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